# Novel Chlorhexidine Releasing System Developed from Thermosensitive Vinyl Ether-Based Hydrogels

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**Abstract: The aim of this study is to determine the effective concentrations of chlorhexidine on the release for prolonged periods of time from a novel hydrogel system. A hydrogel that exhibits a volume phase transition in response to temperature was synthesized by radiation copolymerization of ethylene glycol vinyl ether and butyl vinyl ether in the presence of crosslinking agent, diethylene glycol divinyl ether. Hydrogel samples in the disc form (diameter, 10 mm and height, 1.5 mm) were utilized as a matrix for the release of an antimicrobial agent, chlorhexidine diacetate. Chlorhexidine loading into the hydrogel was performed by water sorption at 4°C, which allows high swelling and thus high loading capacity, i.e., ~36 mg drug per gram of dry gel. Chlorhexidine release was examined as short-term (24 h) and long-term (27 days) by UV spectrophotometer. Microbial studies were carried out by micro-dilution method in order to determine the effectiveness of the drug release. Minimum inhibitory concentration values for the pathogens of *Streptococcus mutans* and *Lactobacillus casei* were determined. The long-term chlorhexidine release is initially very fast. After that, the drug release reaches a slow but a steady rate. Such a release pattern provides an effective drug release. The prolonged release of chlorhexidine is continued up to the 27th day. MIC values for the two pathogens have been shown that the release rate from disc is effective to inhibit the growth of pathogens. These *in vitro* drug release results suggested that the thermosensitive hydrogelic system developed in this study can be evaluated as a delivery system for the release of chlorhexidine.** © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 83B: 609–614, 2007

**Keywords: chlorhexidine; controlled-release; hydrogel; thermoresponsive polymer**

## INTRODUCTION

Chlorhexidine is a broad-spectrum antimicrobial agent and appears to be the most effective antiplaque medicament available today. It is widely used as a mouthrinse in the prevention and treatment of periodontal diseases and dental caries. The value of 0.2% (w/v) chlorhexidine mouthwash used twice daily in 10 mL doses for 1 min is recommended. One of the major indications for its use in dentistry has been the reduction of oral mutans streptococci.[1] However, with the clinical application of chlorhexidine as a mouthrinse, irrigant, or topical agent, some side effects have been noted. Discoloration of teeth, restorations and oral tissues, unpleasant taste, and interference with the sense of taste are common findings.[2] In addition, the applications mentioned earlier require good patient compliance to achieve optimal therapeutic effect. A method for localized and prolonged administration of chlorhexidine for oral diseases would therefore be desirable. The controlled release systems fulfill these requirements by maintaining a fairly constant concentration of the drug for an extended period of time.[3,4] Currently, chlorhexidine is the only antiseptic available for sustained release, local delivery for periodontal therapy.[5] The PerioChip® (manufactured by Perio Products, Jerusalem, Israel, distributed by Astra USA, Westborough, MA) is a small, rectangular chip that contains 2.5 mg chlorhexidine gluconate in a biodegradable gelatin matrix crosslinked with glutaraldehyde. It is placed in periodontal pocket and provides sustained release of chlorhexidine over a 7-day period.[6] However, PerioChip has certain shortcomings including poor handling characteristics and sub-optimal release profiles.[7] Therefore, a number of chlorhexidine sustained-release devices have been developed by several groups for treatment of periodontal

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diseases.[8–11] Some of these devices are made from biodegradable polymers.[8,9] Non-degradable systems are based on ethyl cellulose[10] and ethylene vinyl acetate copolymer.[11] However, it is difficult to formulate a delivery composition for chlorhexidine because of its structural properties described in Discussion.

In this presented study, we investigated the use of thermoresponsive poly(vinyl ether) based amphiphilic hydrogel for the local controlled delivery of chlorhexidine in the oral environment. The aim of developing this novel system is different than commercially available systems such as PerioChip. PerioChip is a small rectangular chip which is placed in periodental pocket and releases relatively small amounts of chlorhexidine. The novel system is developed as an intra oral delivery system that attached to tooth crown for replacing chlorhexidine mouthrinses.

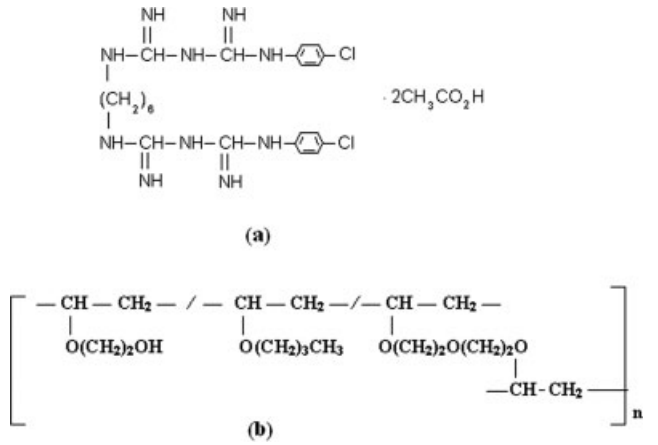


**Figure 1.** Chemical structures of (a) Chlorhexidine diacetate and (b) (EGVE-BVE) hydrogel synthesized in this study.

## MATERIALS AND METHODS

### Chemicals

Comonomers, ethylene glycol vinyl ether (EGVE), butyl vinyl ether (BVE), and crosslinking agent diethylene glycol divinyl ether (DEGDVE) were obtained from Aldrich (Germany) in high purity (>99%) and they were used without further purification. Phosphate buffer solution (PBS) of pH 7.4 was used as swelling medium to determine the temperature sensitivity of the hydrogels. The buffer solution was prepared by using PBS tablets obtained from Sigma (Germany). The antimicrobial agent, chlorhexidine diacetate was obtained from Drogsan (Turkey).

### Synthesis of the Hydrogels

The thermosensitive hydrogels were prepared according to the method used in our earlier studies.[12,13] In brief, the mixture consisting of comonomers in 60:40 mole ratio (EGVE: BVE) and crosslinker (4.0 mol %) were placed into the Pyrex test tube, degassed, and sealed in vacuum. The irradiation copolymerization was carried out on a $^{60}$Co $\gamma$-source at irradiation dose rate of 2.51 kGy $h^{-1}$ during 42 h. After the polymerization the gels were removed from the tubes, cut into discs of $\sim$10 mm in diameter and 1.5 mm thickness. Then the gels were washed in distilled water for $\sim$2 weeks to remove any unreacted monomer. The gels were then put in isopropyl alcohol for several days and later washed out in distilled water. The discs were dried under vacuum at 25°C until they reached a constant weight (its dry weight is $\sim$0.15 g). The chemical structures of synthesized hydrogel and chlorhexidine diacetate are given in Figure 1.

### Chlorhexidine Loading into the Hydrogels

To prepare the chlorhexidine stock solution known amounts of chlorhexidine diacetate were added to a known volume of distilled water and then were incubated in a water bath at 37°C for 2 days with stirring. After equilibration, i.e., no more drug could be dissolved, the chlorhexidine concentration in the solution was assayed by UV–vis spectrophotometry (Hitachi 150-200) following appropriate dilution. The loading solutions in desired concentrations were prepared from the stock solution. Previously dried hydrogel sample in known amount (e.g., 0.15 g) was immersed in 10 mL water with two different drug concentratios, i.e. 1 and 3 mg $mL^{-1}$, at 4°C for 3 days. The remaining chlorhexidine in the solution was then analyzed by spectrophotometer. The amount of loaded drug into the hydrogel was calculated from mass balance for each drug concentration in the solution. Then the loaded gels were dried under vacuum at 25°C until they obtained a constant weight.

### Thermosensitivity of the Hydrogels

To determine the thermosensitivity of hydrogels, dynamic swelling measurements were done by gravimetric means at two different temperatures, i.e. 4 and 37°C. The hydrogel discs were dried to the constant weight in vacuum and then immersed in a constant temperature bath filled with PBS (pH 7.4, $I = 0.2$). The samples were removed from the PBS at appropriate intervals, blotted with filter paper, and weighed by using an analytical balance until the equilibrium was obtained. The water contents (WC) were calculated on dry basis by using the following equation:

$$\text{Water content } (\%) = [(W_s - W_d)/W_d] \times 100 \qquad (1)$$

where, $W_s$ is the swollen weight; $W_d$ is the dry weight of the polymer gels. All the swelling experiments were repeated at least three times and the results were reported as average values.

### *In Vitro* Release Studies

*In vitro* release studies were conducted in a shaker agitating at 50 rpm at 37°C. A dried chlorhexidine loaded hydrogel

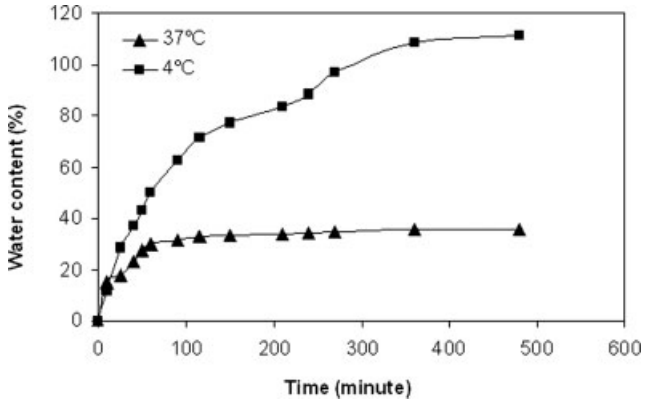


**Figure 2.** Swelling kinetics of (EGVE-BVE) hydrogels at two different temperatures (4°C and 37°C) in PBS (pH 7.4; $I = 0.2$).

disc was placed in a sealed Erlenmayer containing 25 mL of distilled water. At certain time intervals the releasing medium was withdrawn from the Erlenmayer and the medium was replaced with distilled water. The release of chlorhexidine was determined by UV spectroscopy, by the peak height of the 256 nm peak of chlorhexidine diacetate solutions. Then cumulative chlorhexidine release was calculated. Short-term drug release tests lasted for 24 h, with a sampling interval of 30 min. Long-term tests continued for 27 days, with a sampling interval of 24 h. All release studies were repeated three times and the results were reported as average values.

### Antibacterial Activity Tests

The bacteria used in this study are *Streptococcus mutans* (RSKK 676, isolated by Hacettepe University at 1986; type A 10919) and *Lactobacillus casei* (RSKK 706, isolated by Institute Superior di Santa Roma). These strains were obtained from Refik Saydam Central National Institute of Health Culture Collection (Ankara, Turkey). They were cultured on Mueller-Hinton broth. After a 2–5 h incubation at 37°C, the culture was diluted in Mueller Hinton broth to the turbidity of 0.5 McFarland standart ($10^8$ cfu $mL^{-1}$) and was then further diluted to $5 \times 10^5$ cfu $mL^{-1}$.

To test the antibacterial effect of the chlorhexidine-containing discs, they were placed in a 25 mL sealed Erlenmayer containing distilled water at 37°C. At certain time intervals 1 mL sample was withdrawn from the releasing medium and stored at −20°C for microbiologic studies. Antibacterial activities of the samples were determined according to the microdilution method proposed by National Committee for Clinical Standards (NCCLS).[14] The minimum inhibitory concentration (MIC) values were determined after 24 h incubation at 37°C as the lowest concentration of drug showing no visible growth.

## RESULTS

The plots of the water content in the hydrogel versus time were constructed at two different temperatures (4°C and 37°C) at constant pH and ionic strength (pH 7.4; $I = 0.2$) (Figure 2). The temperature increment from 4 to 37°C leads to low swelling values indicating that the temperature decrease favors the uptake of solvent into the gel. Rapid swelling was observed during 60 min. Long-term water content values of the hydrogel were listed in Table I at 4 and 37°C. This table indicates that hydrogel reaches equilibrium at the end of 6 h at 37°C; however, at 4°C equilibrium was established approximately at the end of 24 h.

The drug loading procedure was realized at two concentrations of loading solution, i.e., 1 and 3 mg $mL^{-1}$ chlorhexidine solutions. The amount of loaded chlorhexidine to each hydrogel disc was calculated from the difference between initial and final reservoir concentrations and it was repesented as "milligram chlorhexidine per gram of dry hydrogel." Experiments in which the loading time was varied showed that 3 days period was sufficient for complete loading. The results showed that $\sim 10$ and $\sim 36$ mg chlorhexidine was loaded into the gram of dry gel from the drug solutions of 1 and 3 mg $mL^{-1}$, respectively.

*In vitro* release studies were done only by using the hydrogel discs which were loaded from 3 mg $mL^{-1}$ chlorhexidine solution, since 1 mg $mL^{-1}$ chlorhexidine solution leads to very low amount of chlorhexidine loading, e.g. 10 mg per gram of dry gel. Cumulative amounts of chlorhexidine released from the hydrogel at 37°C as a function of time are shown in Figure 3(a) and (b) for short-term and long-term release studies, respectively. Short-term release was studied during 24 h. The release profile is characterized by an initial burst of chlorhexidine during the first 2 h followed an intermediate releasing rate from 2 to 5 h, and a relatively low release rate from 5 to 24 h [Figure 3(a)]. Figure 3(b) shows the prolonged release of chlorhexidine up to 650 h. Fifty percent of the total drug releases first 4 days, then release rate decreases significantly and steady release lasts about 27 days.

In Figure 4 the fractional release of chlorhexidine F is plotted versus time$^{1/2}$, $t^{1/2}$. After the burst effect period for a short time period of drug release, $0.3 \leq F \leq 0.6$, $F$ is linear in $t^{1/2}$, indicated that the drug delivery shows a Fickian diffusion mechanism and the expression given below is used to determine the apparent diffusion coefficient ($D_s$) for chlorhexidine release from the hydrogel.

$$F = M_t/M_\infty = 4(D_s t/\pi h^2)^{1/2} \quad (2)$$

Here, $M_t$ and $M_\infty$ are the amount of drug released at the time $t$ and the maximum amount of chlorhexidine release,

**TABLE I. Long-Term Water Contents of the Hydrogels at 4°C and 37°C**

| Swelling Period (minute) | Water Content (%) | |
|---|---|---|
| | 4°C | 37°C |
| 360 | 108 ± 5 | 35 ± 1 |
| 1440 | 125 ± 3 | 36 ± 2 |
| 2880 | 128 ± 4 | 37 ± 1 |

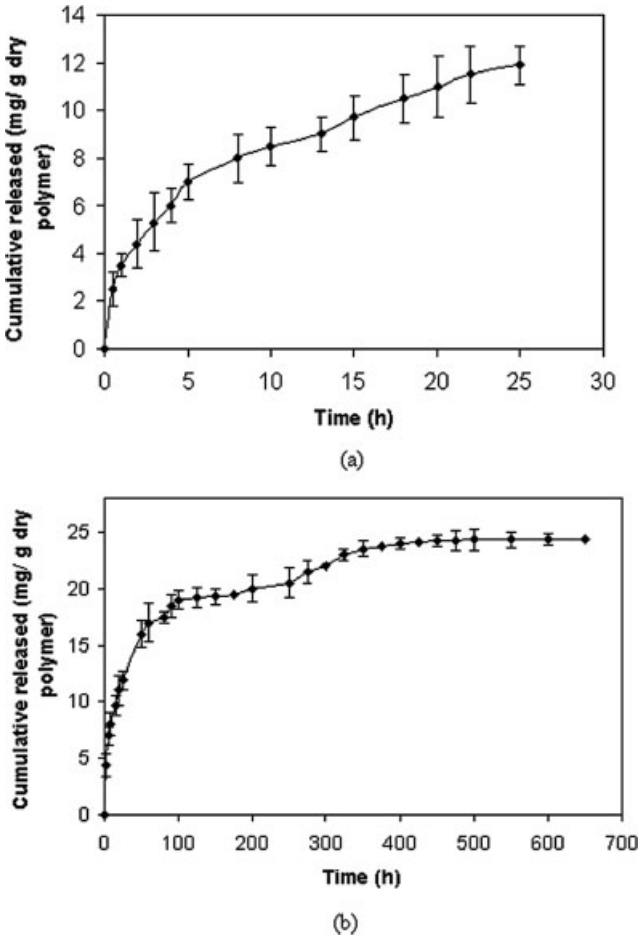


**Figure 3.** The *in vitro* release profiles of chlorhexidine from hydrogels at 37°C in PBS (pH 7.4). (a) Short-term release profile (up to 24 h) and (b) Long-term release profile (up to 650 h).

respectively. $h$ is the thickness of the drug-loaded hydrogel. In accordance with Eq. (2) the slope of this plot yields $D_s = 5.73 \times 10^{-12}$ m$^2$ s$^{-1}$.

The chlorhexidine MICs for the two pathogens were determined as follows: *S. mutans* 0.17–0.25 $\mu$g mL$^{-1}$ and

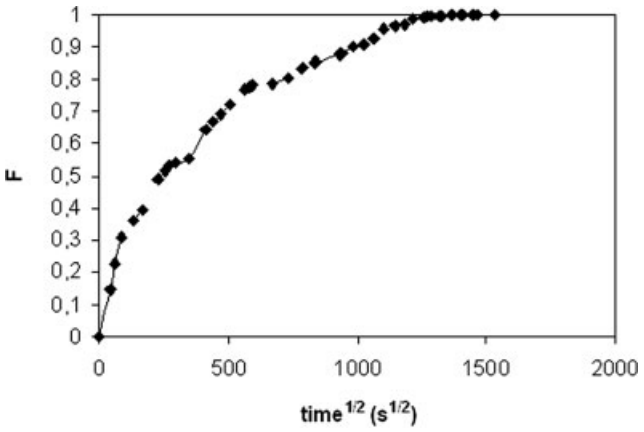


**Figure 4.** Fractional release of chlorhexidine from hydrogels as a function of (time)$^{1/2}$.

**TABLE II. Time-Dependent Released Amounts of Chlorhexidine from (EGVE-BVE) Hydrogels *In Vitro***

| Time (h) | Released Amounts of Chlorhexidine ($\mu$g ml$^{-1}$) |
|---|---|
| 0 | 0 |
| 0.5 | 1.3 ± 0.0 |
| 1.0 | 4.6 ± 0.0 |
| 5.0 | 5.0 ± 0.4 |
| 8.0 | 5.5 ± 0.7 |
| 24.0 | 8.7 ± 0.6 |
| 31.0 | 11 ± 1.2 |
| 55.0 | 14.2 ± 0.5 |
| 78.0 | 15.1 ± 0.5 |
| 96.0 | 15.5 ± 0.6 |
| 112.0 | 17.4 ± 0.9 |
| 161.0 | 15.9 ± 1.6 |
| 181.0 | 15.5 ± 2.3 |
| 230.0 | 19.5 ± 1.0 |

*L. casei*, 0.11–0.14 $\mu$g mL$^{-1}$. Table II shows the average released amounts of chlorhexidine *in vitro*.

## DISCUSSION

In this study, we examined the feasibility of use of a thermosensitive hydrogel as a possible local delivery system for chlorhexidine. The components of this hydrogel are hydrophilic monomer EGVE and hydrophobic comonomer BVE. The crosslinking agent was bifunctional DEGDVE. The hydrogel was successfully synthesized by $\gamma$-irradiation. The details of synthesis and characterization of the hydrogel were given in our previous publications.[12,13] In one of these studies (EGVE-BVE) hydrogels were used in cultivation of human skin fibroblasts (HS $An_1$). The results showed that the cells can attach and proliferate on the surface of thermoresponsive polymer. No cytotoxicity was observed towards fibroblast cells *in vitro*. Neither cell death nor growth disorder was observed throughout the culture period.[13]

Chlorhexidine is highly cationic, it exhibits high reactivity, and thus strongly binds especially with the ionic polymeric carriers. This property leads to the undetectable release of chlorhexidine. As a consequence, polymeric carriers used for delivery of other antimicrobial agents become unsuitable for chlorhexidine.[10] The hydrogelic system developed in our study contains non-ionizable groups (Figure 1). This is why, it offers a great advantage as a chlorhexidine release device.

In our previous study swelling experiments were done at four different temperatures i.e. 4, 10, 25, and 37°C. The results indicated that the copolymer of EGVE and BVE in the molar ratio 60/40 (in feed) containing both hydrophilic and hydrophobic groups should exhibit thermosensitivity owing to the strengthening of the hydrophobic interactions

with increasing temperature.[12] It means, this hydrogel exhibits a lower critical solution temperature (LCST) behavior or in other words, an inverse (or negative) temperature-dependence.

In this study, we consider the usability of thermoresponsive behaviour of hydrogel for chlorhexidine loading. As indicated in relevant literature, the most common drug loading methods are solvent sorption (or embedding) technique and loading during polymerization/crosslinking method.[15] Here, chlorhexidine loading into crosslinked hydrogel was performed by water sorption. By taking into account the temperature-dependent water sorption property of the hydrogel, chlorhexidine was loaded at low temperature, i.e., 4°C, which allows high swelling and thus high loading capacity.

As expected, the amount of loaded chlorhexidine was increased as the concentration of loading solution increased. Since the solubility of chlorhexidine in water is 3 mg $mL^{-1}$ the highest amount of loadable drug is not exceed 36 mg per gram of dry gel. It means one disc contains 5.4 mg chlorhexidine because its dry weight is about 0.15 g. This is why, it can be concluded that our hydrogel device has twofold higher loading capability than commercially available product, PerioChip (contains $\sim$2.5 mg chlorhexidine). It is also possible to adjust the loading capacity of the device by changing the dimensions of the disc.

The initial burst effect which is seen in short-term release curve [Figure 3(a)] may be attributed to the localization of drug molecules closer to the surface of the hydrogel during loading and drying processes. After the embedding procedure, drying of the loaded hydrogels starts at the surface and water from the interior of the hydrogel is pulled along with the drug to the surface by capillary forces in the absence of specific polymer–drug interaction. At the end of 24 h, 34% of loaded drug was released.

Release pattern in Figure 3(b) seems desirable because the initial rapid release is beneficial to establishing an effective drug level in the solution; and the following slow but steady release is appropriate for maintaining the drug concentration at the therapeutic level. At the end of 25 days, $\sim$70% of the total loaded drug has been released. It means, about 3.8 mg chlorhexidine is released from the device at the end of the release period under study. Huang et. al. have reported relatively low amount of released chlorhexidine (1.6 mg drug release at the end of 25 days).[10]

The apparent diffusion coefficient obtained was $D_s = 5.73 \times 10^{-12}$ $m^2$ $s^{-1}$. Since the $D_s$ changes significantly with crosslinker concentration in the gel, its magnitude indicates the rate of drug release e.g. increasing the crosslinking density reduces $D_s$, which means a slower drug release. The $D_s$ value obtained here is a reasonable value for a hydrogel and it is consistent with the others findings.[16,17]

*S. mutans* is considered one of the most important cariogenic species of the human oral microbial flora.[18] Although the role of *Lactobacillu* in the carious process is not well defined, it is believed that, they are involved more in the progression of the deep enamel lesion rather than initiation. They are the pioneer organisms in the advancing front of carious process, especially in dentin.[19] Hennessey[20] reported that the MIC value of *S. mutans* to chlorhexidine was 0.19 $\mu$g $mL^{-1}$. Jarvinen et al.[14] showed that chlorhexidine was highly effective against all the *S. mutans* isolates and its MIC values did not exceed 1 $\mu$g $mL^{-1}$ (in the range between 0.25 and 1 $\mu$g $mL^{-1}$). We found similar chlorhexidine MICs for *S. mutans* between 0.17 and 0.25 $\mu$g $mL^{-1}$. In the case of *L. casei*, MICs varies between 0.11 and 0.14 $\mu$g $mL^{-1}$.

Since all the released amounts of the chlorhexidine from the hydrogel during the 230 h releasing period (Table II) exceeded the MIC for both microbial flora, *S. mutans* and *L. casei*, the chlorhexidine releasing device developed in this study appeared to be an effective vehicle for the controlled delivery of drug.

Medlicott et al.[21] reported that 8 h after administration of a 0.2% w/v chlorhexidine mouthrinse, the measured chlorhexidine concentrations in the saliva film by HPLC are between 10 and 27 $\mu$g $mL^{-1}$. The main drawbacks of mouthrinses are bitter taste imparted by the high drug concentration and tooth discolouration with prolonged use. In our system, released amounts vary between 1.3 and 19.5 $\mu$g $mL^{-1}$ during 230 h releasing period. Therefore, it overcomes the drawbacks of mouthrinsing administration by employing smaller quantities of chlorhexidine and delivering the drug to specific sites in the mouth.

## CONCLUSION

In conclusion, the temperature-sensitive vinyl ether-based hydrogel prepared by radiation copolymerization has attractive properties as chlorhexidine releasing matrix. The temperature-dependent swelling ability of the hydrogel will become an important factor for selection of this material for high-loading efficiency. Since the hydrogel contains non-ionizable groups there is no strong binding between drug and polymer. Thus, detectable release is obtained and drug release kinetics are reproducible. The chlorhexidine released from the experimental hydrogel device has a clearly inhibitory effect with a safe dose on the cariogenic bacteria tested. Thus the *in vitro* controlled release delivery system tested appeared to be an effective vehicle for the application chlorhexidine to a localized area such as possibly to the surfaces of tooth or restoration. Since the present chlorhexidine mouthrinses require good patient compliance to achieve optimal therapeutic effect, this intraoral delivery system could be beneficial for individuals with mental or physical handicaps, immunsupressed, and palliative care patients that compromise oral hygiene. Also, it would be useful for patients with restricted mandibular movements (Trismus) and during the fixation period of mandibular fractures.

## REFERENCES


1. Gjermo P. Chlorhexidine and related compounds. J Dent Res 1989;68(Special Issue):1602–1608.
2. Loe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and ginivitis in man. J Periodont Res 1970;5:79–83.
3. Schwach-Abdellaoui K, Vivien-Castioni N, Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur J Pharm Biopharm 2000;50:80–93.
4. Vyas SP, Sihorkar V, Mishra V. Controlled and targeted drug delivery strategies towards intraperiodontal pocket diseases. J Clin Pharm Therapeutics 2000;25:21–42.
5. Ciancio GS. Antiseptics and antibiotics as chemotherapeutic agents for periodontitis management. Compendium 2000;21:59–76.
6. PerioChip package insert, Astra Pharmaceuticals, 1998.
7. Yue IC, Poff J, Cortes ME, Sinisterra RD, Faris CB, Hildgen P, Langer R, Shastri VP. A novel polymeric chlorhexidine delivery device for the treatment of periodontal disease. Biomaterials 2004;25:3743–3750.
8. Heling I, Steinberg D, Kenig S, Gavrilovich I, Sela MN, Friedman M. Efficacy of a sustained-release device containing chlorhexidine and $Ca(OH)_2$ in preventing secondary infection of dentinal tubules. Int Endod J 1992;25:20–24.
9. Şenel S, İkinci G, Kaş S, Yousefi-Rad A, Sargon MF, Hıncal A. Chitosan films and hydrogels for chlorhexidine gluconate for oral mucosal delivery. Int J Pharm 2000;193:197–203.
10. Huang J, Wong HL, Zhou Y, Wu XY, Grad H, Komorowski R, Friedman S. In vitro studies and modeling of a controlled-release device for root canal therapy. J Controlled Release 2000;67:293–307.
11. Lin DM, Kalachandra S, Valiyaparambil J, Offenbacher S. A polymeric device for delivery of anti-microbial and anti-fungal drugs in the oral environment: Effect of temperature and medium on the rate of drug release. Dent Mater 2003;19:589–596.
12. Gümüşderelioğlu M, Müftüoğlu O, Karakeçili AG. Biomodification of thermosensitive copolymer of ethylene glycole vinyl ether by RGD and insulin. React Funct Polym 2004;58:149–156.
13. Gümüşderelioğlu M, Karakeçili AG. Uses of thermoresponsive and RGD/insulin-modified poly(vinyl ether)-based hydrogels in cell cultures. J Biomater Sci Polymer Edn 2003:14(3): 199–211.
14. Jarvinen H, Tenovuo J, Huovienen P. In vitro susceptibility of *Streptocus mutans* to chlorhexidine and six other antimicrobial agents. Antimicrobial Agents Chemother 1993;37:1158–1159.
15. Ramkissoon-Ganorkar C, Liu F, Baudys M, Kim SW. Modulating insulin-release profile from pH/thermosensitive polymeric beads through polymer molecular weight. J Controlled Release 1999;59:287–298.
16. Shah SS, Cha Y, Pitt CG. Poly (glycolic acid-*co*-DL-lactic acid): Diffusion or degradation controlled drug delivery? J Controlled Release 1992;18:261–270.
17. Teijon JM, Trigo RM, Garcia O, Blanco MD. Cytarabine trapping in poly(2-hydroxyethyl methacrylate) hydrogels:drug delivery studies. Biomaterials 1997;18:383–388.
18. Spets-Happonen S, Markkanen H, Pöllanen L, Kauppinen T, Luoma H. Salivary *Streptococcus mutans* count and gingivitis in children after rinsing with a chlorhexidine-fluoride solution with and without strontium. Scand J Dent Res 1985;93:329–335.
19. Samaranayake LP.Microbiology of dental caries. In: Essential Microbiology for Dentistry, 2nd ed. New York: Churchill Livingstone; 2002. pp 217–223.
20. Hennessey TD. Some antimicrobial properties of chlorhexidine. J Periodontal Res 1973;8(Suppl. 12):61–67.
21. Medlicott NJ, Ferry DG, Tucker IG, Rathbone MJ, Holborow DW, Jones DS. High performance liquid chromatographic (HPLC) assay for the determination of chlorhexidine in saliva film. J Liq Chromatog 1994;17(7):1605–1620.